SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 16, No. 10, pp. 2179–2186, 1997
Printed in the USA
0730-7268/97 $6.00 + .00

A COMPARISON OF EQUILIBRIUM PARTITIONING AND CRITICAL BODY RESIDUE APPROACHES FOR PREDICTING TOXICITY OF SEDIMENT-ASSOCIATED FLUORANTHENE TO FRESHWATER AMPHIPODS

SUSAN KANE DRISCOLL† and PETER F. LANDRUM*‡

†Cooperative Institute for Limnology and Ecosystem Research, University of Michigan, Ann Arbor, Michigan 48109, USA
‡National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, 2205 Commonwealth Boulevard, Ann Arbor, Michigan 48105-1593, USA

(*Received* 23 *September* 1996; *Accepted* 4 *April* 1997)

Abstract—Equilibrium partitioning (EqP) theory, which has been used to develop sediment quality criteria, predicts that the effects of organic compounds in sediments can be assessed by comparison of organic carbon-normalized sediment concentrations and estimated pore-water concentrations to effects determined in water-only exposures. A complementary approach, the critical body residue (CBR) theory, examines actual body burdens in relation to toxic effects. Critical body residue theory predicts that the narcotic effects of nonpolar compounds should be essentially constant for similar organisms, and narcosis should be observed at body burdens of 2 to 8 μmol/g tissue. This study compares these two approaches for predicting toxicity of the polycyclic aromatic hydrocarbon (PAH) fluoranthene. The freshwater amphipods *Hyalella azteca* and *Diporeia* spp. were exposed for up to 30 d to sediment spiked with radiolabeled fluoranthene at concentrations of 0.1 (trace) to 3,940 nmol/g dry weight (=346 μmol/g organic carbon). Mean survival of *Diporeia* was generally high (>70%) and not significantly different from that of control animals. This result agrees with EqP predictions, because little mortality was observed for *Diporeia* in 10-d water-only exposures to fluoranthene in previous studies. After 10-d exposures, mortality of *H. azteca* was not significantly different from that of controls, even though measured interstitial water concentrations exceeded the previously determined 10-d water-only median lethal concentration (LC50). Equilibrium partitioning overpredicted fluoranthene sediment toxicity in this species. More mortality was observed for *H. azteca* at later time points, and a 16-d LC50 of 3,550 nmol/g dry weight sediment (291 μmol/g organic carbon) was determined. A body burden of 1.10 μmol fluoranthene-equivalents/g wet weight in *H. azteca* was associated with 50% mortality after 16-d exposures. Body burdens as high as 5.9 μmol/g wet weight resulted in little mortality in *Diporeia*. *Diporeia*, which has limited ability to metabolize fluoranthene and a higher lipid content, appears to be less sensitive than *H. azteca*, which does metabolize fluoranthene. These results demonstrate that the CBR approach is a useful complement to the EqP approach for the prediction and assessment of toxicity associated with contaminated sediments.

Keywords Equilibrium partitioning Critical body residue Fluoranthene Sediment Amphipods

INTRODUCTION

Predicting the fate and effects of persistent, sediment-associated organic contaminants continues to be an important element in ecological and human health-based risk assessment. One approach, equilibrium partitioning (EqP), has been used by the U.S. Environmental Protection Agency (U.S. EPA) to develop sediment quality criteria (SQC) for a suite of polycyclic aromatic hydrocarbons and pesticides [1]. Equilibrium partitioning predicts the biological effects of hydrophobic compounds on the basis of their organic carbon-normalized concentration in the sediment and estimated pore-water concentrations. Because the chemical activity of a compound in sediment will be reflected by its freely dissolved interstitial water concentration, effects should be observed when interstitial water concentrations exceed concentrations that result in toxicity in water-only exposures. Toxicity bioassays with benthic invertebrates have, in general, confirmed the utility of the approach [2,3]. However, exceptions have been noted with certain species and sediments [4–7].

A complementary approach for evaluating contaminant exposure, the critical body residue (CBR) approach, measures actual body burdens of a compound in relation to toxic effects [8]. This approach predicts that, for chemicals that act by narcosis (most nonpolar organics), the potency measured at the site of toxic action should be essentially constant for similar organisms [8,9]. One advantage of the CBR approach is that differences in susceptibility between organisms can be directly assessed without confounding elements such as rate of accumulation or bioavailability. The CBR method has been validated with a variety of aquatic vertebrates [10–13] and invertebrates [5,6,14,15].

A recent study [7] compared the sensitivity of the standard freshwater amphipod test species *Hyalella azteca* [16] to the benthic amphipod *Diporeia* spp. [17], based on actual body burdens and observed toxic effects of sediment-associated fluoranthene. Fluoranthene was chosen as a compound of interest because the U.S. EPA has proposed an SQC for this compound of 620 μg fluoranthene/g sediment organic carbon (3.06 μmol/g organic carbon) [18]. In these previous sediment experiments, *Diporeia* accumulated more fluoranthene and exhibited greater mortality than did *H. azteca* [7]. This result did not support the EqP approach, because in water-only exposures, *H. azteca* was shown to be more sensitive to fluoranthene than was *Diporeia* [19]. For both species, dose based on actual body burden was generally a more reliable predictor of toxicity than was EqP.

The present study represents a continuation of previous efforts to test the ability of the EqP and CBR approaches to

* To whom correspondence may be addressed.

Table 1. Concentration and purity of fluoranthene in sediment

Organism	Nominal sediment concn.[a]	Mean (SD) measured sediment concn.[a] (n = 3), day 2	Mean (SD) measured sediment concn.[a] (n = 5), day 30	Overall mean (SD) measured sediment concn.[a] (n)	Measured sediment concn.[a] GC-MS (n = 1) day 30[b]	Mean % purity (n = 2) day 30[c]
Diporeia spp.	0.308	0.171 (0.004)	0.170 (0.014)	0.165 (0.22) (16)	ND	81.8
	492	587 (89)	583 (67)	607 (91) (16)	587	88.2
	986	799 (120)	1,080 (379)	898 (266) (16)	1,220	91.3
	1,940	2,160 (312)	2,510 (329)	2,270 (294) (15)	2,090	86.8
	3,910	4,250 (1,650)	1,820 (1,020)	2,800 (2,170) (15)	4,250	96.2
Hyalella azteca	0.308	0.173 (0.009)	0.132 (0.017)	0.152 (0.02) (16)	ND	89.3
	492	564 (39)	537 (137)	563 (101) (15)	618	89.8
	986	1,280 (224)	770 (418)	1,040 (364) (16)	1,300	89.5
	1,940	2,080 (595)	2,180 (777)	1,850 (677) (16)	3,430	95.8
	3,910	3,170 (264)	4,670 (2,940)	3,940 (2,190) (16)	5,250	96.6

[a] nmol/g dry wt.
[b] GC-MS = gas chromatography–mass spectrometry, ND = not determined.
[c] Percent of total extracted radioactivity that comigrated with a pure fluoranthene standard on thin-layer chromatography.

assess the bioavailability and toxicity of contaminants. Specifically, this work tests whether the toxic effects of a sediment-associated, hydrophobic organic contaminant (fluoranthene) can be predicted on the basis of its 10-d water-only median lethal concentration (LC50) and sediment organic carbon-normalized concentration. This work also tests whether toxicity can be predicted on the basis of observed CBRs of 2 to 8 μmol fluoranthene/g wet weight tissue. This work extends the scope of past assessments by testing for differences in sediment properties, specifically sediment organic carbon content. These experiments repeat the experimental design of a previous study [7], using sediment with a higher organic carbon content (1.29% vs 0.37%). The development of appropriate and useful national, chemical-specific SQC requires repeated testing of the assumptions of the underlying methods and the ability of the approaches to accurately predict the bioavailability and effects of organic contaminants for a variety of sediment types.

MATERIALS AND METHODS

Chemicals

Radiolabeled [3-^{14}C]fluoranthene was purchased from Sigma Chemical Co. (St. Louis, MO, USA) with a specific activity of 45 mCi/mmol. Unlabeled fluoranthene was purchased from Aldrich Chemical (Milwaukee, WI, USA). The [^{14}C]fluoranthene was determined to be >98% pure by thin-layer chromatography (hexane : benzene, 80:20, v/v) on silica plates (Alltech, Deerfield, IL, USA) and liquid scintillation counting (LSC).

Separate stock solutions were prepared for each sediment concentration by combining [^{14}C]fluoranthene with unlabeled fluoranthene in acetone. The concentration of total fluoranthene in each stock solution was determined by gas chromatography–mass spectrometry–selected ion monitoring (GC-MS-SIM) using a Hewlett Packard model 5980 series II gas chromatograph equipped with a model 5971 mass selective detector (Hewlett Packard, San Fernando, CA, USA) [20]. The concentration of radiolabeled fluoranthene in each stock solution was determined by LSC on a Tri-Carb Liquid Scintillation Analyzer (Model 2500 TR, Packard Instrument, Meridien, CT, USA). Samples were corrected for quench using the external standards ratio method after subtracting background. Triplicate samples from each stock solution were analyzed by LSC and the mean value was used to calculate a specific activity for each stock solution (μCi of radiolabeled fluoranthene/μmol of total fluoranthene determined from GC-MS).

Sediment spiking

Sediment was collected in May 1995 from a 100-m-deep site in Lake Michigan and sieved (<1 mm) to remove debris and macrofauna. Average organic carbon content of the sediment, analyzed prior to spiking with fluoranthene and measured on a model 2400 CHN Elemental Analyzer (Perkin Elmer, Norwalk, CT, USA) after acidification to remove carbonates, was 1.14% (SD = 0.09, n = 7). Nominal concentrations of fluoranthene in this experiment, calculated to be equivalent to concentrations used in two previous experiments on an organic carbon-normalized basis [7], ranged from 0.308 to 3910 nmol/g dry weight (Table 1) and from 0.027 to 343 μmol/g organic carbon.

Sediments were spiked on June 14, 1995, as previously described [7] using a standard rolling jar method [21]. Briefly, stock solutions of fluoranthene in acetone were evaporated onto the inside of 1-gallon (3.785-L) glass jars. Sediment (2,400–2,600 g wet weight) and filtered (0.45 μm) Huron River water (150 ml) were added to the jars, and the slurry was rolled at room temperature for 2 h, held overnight at 4°C, and rolled again at room temperature for 2 h. Because crystallized compound could still be seen on the insides of the glass jars, the sediments were rolled again the following day for 8 h. Triplicate sediment samples from each concentration were taken for LSC, dry to wet weight determinations, and to determine the thoroughness of mixing. Sediments were held to equilibrate at 4°C for 60 d to allow for dissolution and partitioning of spiked fluoranthene to occur. Immediately prior to the start of the experiment, sediments were rolled again for 6 h to incorporate overlying water that had exuded from the sediment during storage. Five replicate sediment samples were taken from each concentration for LSC, dry to wet weight determination, and to determine thoroughness of mixing. Triplicate sediment samples from each concentration were taken for organic carbon determination on day −2 of the experiment.

Exposure

Diporeia were collected in August 1995 by ponar grab from a previously described 29-m deep station in Lake Michigan that has low concentrations of PAHS in the sediment [22].

Hyalella azteca were obtained in August 1995 from C. Ingersoll at the National Biological Survey in Columbia, Missouri, USA. *Hyalella azteca* were of a size that passed through a 1-mm sieve, but were retained on a 500-μm sieve (approximately 2–3 weeks old). Animals were acclimated to local water before the start of the experiment. Filtered water (0.45 μm) from the nearby Huron River, which closely matches Lake Michigan water in terms of hardness, alkalinity, and pH (see the Results) was used throughout the experiment. Samples of animals were taken at the start and end (day 30) of the experiment for determination of lipid content by a microgravimetric method [23].

Sediment (100 g wet weight) was added to each 300-ml exposure beaker, 250 ml water was added, and the sediment was allowed to settle for 1 d prior to the addition of animals. Ten animals were added per beaker on day 0. Beakers were randomly placed into water renewal systems [24], which exchanged approximately one third of the overlying water twice per day. Overlying water quality characteristics, including temperature, hardness, alkalinity, pH, and dissolved oxygen were measured as previously described [7]. *Hyalella azteca* were initially fed 1.0 ml of yeast/alfalfa/trout chow (YCT) food per beaker, per day, according to U.S. EPA guidelines [16]. When scum appeared on the sediment surface in beakers containing *H. azteca* on day 10, feeding was reduced to 0.5 ml YCT per day. *Diporeia* were not fed [17]. Experiments were run at 4°C for *Diporeia* and at room temperature (approx. 20°C) for *H. azteca*.

Triplicate beakers were sieved and sampled from each concentration (except controls) on days 1, 2, 5, and 16, and from five replicate beakers on days 10 and 30 (including controls) [7]. Sediment samples were taken from each beaker for wet to dry weight determination and measurement of [^{14}C]fluoranthene concentration by LSC. Percent survival was calculated on the basis of the number of live animals recovered divided by the total number of animals added to each beaker. Death was defined by the absence of all movement when examined under a dissecting microscope. After determination of wet weight, animals were transferred to scintillation cocktail, and concentration of [^{14}C]fluoranthene was determined by LSC. For all samples, concentration of total fluoranthene (radiolabeled and nonlabeled) was calculated on the basis of the measured specific activities of the stock solutions used to spike the sediment. Concentrations are reported as total fluoranthene-equivalents on a molar basis. Growth rates of both species were calculated from the regression of the natural log of wet weight versus exposure time.

On day 10, samples were taken for determination of fluoranthene concentrations in pore water. Subsurface sediment samples, taken from beakers in which all 10 animals were recovered from the top 1 to 2 cm, were centrifuged (30 min at 1,200 *g*) in stainless steel bottles to pellet the sediment, followed by a high speed spin (30 min at 20,000 *g*) to collect the pore water and remove larger colloids. Previous work in our lab with fluoranthene-spiked sediments demonstrated that surface and subsurface organic carbon-normalized sediment concentrations are similar [25]. Radioactivity associated with the supernatant was determined by LSC. Aliquots of the remaining supernatants were passed through C18 Sep-Pak® cartridges (Millipore, Bedford, MA, USA) using a reverse-phase separation method for determining the binding of compound to dissolved organic matter in the pore water [7,26]. Radioactivity that passes through the column without binding presumably represents fluoranthene that was complexed with dissolved organic carbon. The amount of radioactivity that bound to the column, presumably representing freely dissolved fluoranthene, was determined by difference. The concentration of dissolved organic carbon in the supernatant was measured on a Shimadzu Total Organic Carbon Analyzer (TOC-5000, Shimadzu, Kyoto, Japan).

At the end of the experiment, sediment samples were taken from each dose for analysis of organic carbon content. Purity of the fluoranthene was determined by extraction and comparison to a pure fluoranthene standard on thin-layer chromatography (TLC) [7]. The concentration of fluoranthene in sediment samples was determined by GC-MS [7].

Statistics

Linear and nonlinear regression and probit analyses were performed with *SAS/STAT, Version 6*, 4th edition (SAS Institute, Cary, NC, USA). Mortality data were also analyzed with the trimmed Spearman Karber method using *Statistical Methods and Software for Toxicological Data Analysis* (B.A. Zajdlik, University of Waterloo, ON, Canada, and M.C. Newman, Savannah River Ecology Lab, Aiken, SC, USA). Student's *t* test was used when comparing percent survival (arcsine-transformed data), means, or slopes of regression lines. Differences (two-tailed *t* tests) were considered significant when $p < 0.05$. Confidence limits for LD50s were determined according to a recommended method [27].

Modeling

Accumulation of fluoranthene was modeled using a previously described general model [28]

$$\frac{dC_a}{dt} = (k_s \cdot C_s^0 \cdot e^{-\lambda t}) - (k_d \cdot C_a)$$

where C_a is the concentration of fluoranthene in the animal (nmol/g wet weight), k_s is the conditional uptake clearance rate (g dry weight sediment cleared of fluoranthene/g wet weight tissue/d), C_s^0 is the initial concentration in the sediment (nmol/g dry weight), λ is the rate constant (d^{-1}) for reduction in the bioavailable fraction of fluoranthene in sediment, k_d is the rate constant for the elimination of fluoranthene from the animal (d^{-1}), and t is time (d). For these experiments, uptake clearance rates (k_s) were fit by nonlinear regression to the integrated form of the general model

$$C_a = \frac{(k_s C_s^0) \cdot (e^{-\lambda t} - e^{-k_d t})}{k_d - \lambda}$$

An average value of 0.0648/d, determined for the elimination of fluoranthene by *Diporeia* in the presence of sediment [19], was used in modeling *Diporeia* data from this experiment.

RESULTS

Test conditions

Alkalinity, hardness, and pH in the overlying water were not significantly different between experiments, and overall average (SD, $n = 18$) values were 261 (3.2) mg/L total alkalinity as calcium carbonate, 163 (2.5) mg/L total hardness as calcium carbonate, and a pH of 8.3 (0.05), respectively. Average (SD, $n = 18$) temperature and dissolved oxygen for *Diporeia* were 4.0°C (0.1) and 12.0 mg/L (0.1), respectively. Average (SD, $n = 18$) temperature and dissolved oxygen for *H. azteca* were 20.6°C (0.1) and 6.4 mg/L (0.4), respectively.

Table 2. Concentrations of fluoranthene and dissolved organic carbon (DOC) in interstitial water (IW)

Organism	Mean measured sediment concn.[a] (μmol/g organic carbon)	Estimated freely dissolved fluoranthene IW concn.[a,b] (nmol/L)	Measured total fluoranthene IW concn.[c] (day 10) (nmol/L) (n = 1)	Measured freely dissolved fluoranthene IW concn.[d] (nmol/L) (n = 1)	Mean (SD) measured DOC IW concn. (mg/L) (n = 2)
Diporeia spp.	0.014	0.140	0.180	0.076	8.3 (3.4)
	52.3	523	869	616	9.8 (0.9)
	77.4	774	1,380	673	11.5 (6.6)
	196	1,960	2,600	1,350	5.9 (0.2)
	241	2,410	4,260	2,540	7.1 (1.0)
Hyalella azteca	0.012	0.120	0.153	0.014	8.1 (3.3)
	46.1	461	684	469	13.9 (6.7)
	85.2	852	2,070	1,480	6.1 (0.2)
	152	1,520	3,050	2,170	7.1 (0.7)
	323	3,230	5,870	1,760	4.1 (3.5)

[a] Calculated with overall average organic carbon contents.
[b] Calculated with a K_{oc} value of 10^5 and overall mean measured sediment concentrations.
[c] Freely dissolved fluoranthene plus fluoranthene complexed to DOC.
[d] (Measured total fluoranthene interstitial water concentration) · (measured percent freely dissolved fluoranthene).

Average (SD, n = 6) values for ammonia were 32.2 (6.0) and 24.2 (10.4) μg N/L for experiments with *Diporeia* and *H. azteca*, respectively.

Sediment

Average coefficients of variation for sediment concentrations measured immediately after dosing ranged from 7.5 to 92.9% and tended to be greatest at the highest sediment concentrations (data not shown). Coefficients of variation measured after additional mixing, but before the start of the exposure, were lower, ranging from 2.5 to 5.4% at trace concentrations and 28.6 to 38.7% at the highest doses. Comparison of sediment concentrations measured during the experiment on day −2 versus day 30 showed declines in average sediment concentration with time in 5 out of 10 sediment concentrations (Table 1). However, variability in measured sediment concentrations may have contributed to apparent decline in some cases. Therefore, overall sediment concentrations were determined from averages of all sediment concentrations measured during the course of the experiment (days −2 to 30, $n \geq 15$ samples per dose, Table 1). Overall average sediment concentrations generally agreed with nominal concentrations (72–123% of nominal concentrations), excluding trace concentrations, which tended to be lower (49–54% of nominal concentrations). Concentrations determined by GC-MS at the end of the experiment were within 97 to 156% of nominal concentrations and within 92 and 175% of overall mean concentration measured by LSC (Table 1). Mean percent purity of sediment fluoranthene at the end of the experiment (estimated from the percent of total extracted radioactivity that comigrated with a pure fluoranthene standard on TLC) ranged from 82 to 97% (Table 1).

Average (SD, n = 15) organic carbon contents were 1.27% (0.18) and 1.32% (0.13) on day −2, and 1.05% (0.1) and 1.11% (0.12) on day 30 for *Diporeia* and *H. azteca*, respectively. Overall average (SD, n = 30) organic carbon contents (days −2 and 30) were 1.16% (0.18) and 1.22% (0.16) for *Diporeia* and *H. azteca*, respectively. Mean dissolved organic carbon (DOC) concentrations in the interstitial water ranged from 4.1 to 13.9 mg/L (Table 2). For concentrations other than trace level, mean measured sediment organic carbon-normalized fluoranthene concentrations (calculated with the overall average organic carbon contents and overall average sediment concentrations) ranged from 70 to 121% of nominal organic carbon-normalized sediment concentrations (calculated with an organic carbon content of 1.14%, measured before spiking). Measured concentrations of total fluoranthene in interstitial water (both freely dissolved and DOC-complexed) ranged from 128 to 243% of interstitial water concentrations that were estimated on the basis of mean measured organic carbon-normalized sediment concentrations and a log organic carbon partition coefficient (K_{oc}) of 5.0 (Table 2). Estimates of the concentration of fluoranthene in the interstitial water that was freely dissolved typically ranged from 30 to 71% of the total measured interstitial water concentration (freely dissolved plus DOC-complexed fluoranthene), although one sample was lower (9%) (Table 2).

Mortality

Average percent survival of *Diporeia* was greater than 90% after 10-d exposures, and generally greater than 70% at later time points, with no significant difference between controls (or trace level, which served as a mortality control at day 16) and animals exposed to higher concentrations of sediment fluoranthene (Table 3). Average survival in *H. azteca* was also high, greater than 80% after 10-d exposures, but decreased at later time points. After 16- or 30-d exposures, percent survival of *H. azteca* declined significantly at higher sediment concentrations compared to controls. Probit analyses for *H. azteca* produced a 16-d LC50 of 3,550 nmol fluoranthene/g dry weight sediment, with 95% confidence limits of 2,520 to 7,160 nmol/g dry weight. An LC50 could not be estimated for the 30-d exposures, presumably because percent survival did not exhibit a monotonic decline with increasing sediment concentration.

Bioaccumulation

Body burdens of fluoranthene in *Diporeia* typically increased over the first 10 d of exposure, and appeared to approach steady state after 16 d of exposure (Fig. 1). *Diporeia* attained average body burdens as high as 5.9 μmol/g wet weight at higher sediment concentrations. In contrast, body burdens of fluoranthene in *H. azteca* remained low, at about 1 μmol/g wet weight (Fig. 1). The relationship between percent survival of *H. azteca* and body burden of surviving amphipods (taken from all concentrations), yielded a significant linear

Table 3. Mean (SD) percent survival calculated as (number of live animals recovered/number of animals exposed) · 100. Trace level sediment concentrations serve as controls for mortality on day 16

Organism	Mean measured sediment concn.[a]	Day 10 (n = 5)	Day 16 (n = 3)	Day 30 (n = 5)
Diporeia spp.	Control	100 (0)	ND[b]	94 (5.5)
	0.165	100 (0)	97 (5.8)	96 (5.5)
	607	94 (5.5)	97 (5.8)	82 (8.4)
	898	98 (4.5)	93 (5.8)	88 (13)
	2,270	96 (8.9)	93 (5.8)	84 (8.9)
	2,800	90 (7.1)	70 (20)	84 (16.7)
Hyalella azteca	Control	96 (8.9)	ND	88 (8.4)
	0.152	98 (4.5)	100 (0)	88 (8.4)
	563	90 (10)	97 (4.6)	88 (13)
	1,040	98 (4.5)	83 (5.8)[c]	86 (11.4)
	1,850	78 (8.4)	67 (5.8)[c]	46 (32.1)[c]
	3,940	80 (12.2)	50 (0)[c]	52 (13)[c]

[a] nmol/g dry wt.
[b] ND = not determined.
[c] Significantly different from control ($p < 0.05$).

regression for the 16-d exposure (Fig. 2), but not for the 30-d exposure. From this relationship, the body burden associated with 50% mortality (16-d LD50) was estimated to be 1.10 µmol fluoranthene-equivalents/g wet weight.

Apparent steady state biota–sediment accumulation factors (BSAFs, lipid and sediment organic carbon-normalized accumulation factors) were calculated using body burdens measured after 16-d and 30-d exposures for both species (Table 4). Maximum BSAFs were generally higher for *Diporeia* (1.33) than for *H. azteca* (0.667). As seen in previous experiments [7] highest BSAFs were observed at intermediate sediment concentrations. In contrast to previous experiments in

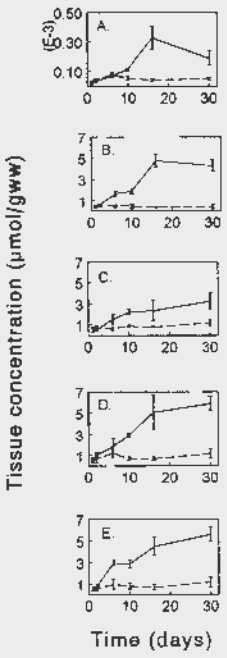

Fig. 1. Concentration of fluoranthene-equivalents in tissue of *Diporeia* (solid lines) and *Hyalella azteca* (dashed lines) over time after exposure to fluoranthene in sediment at nominal concentrations of (A) 0.1 nmol/g dry weight, (C) 986 nmol/g dry weight, and (E) 3,910 nmol/g dry weight. Error bars represent standard deviations of three to five samples.

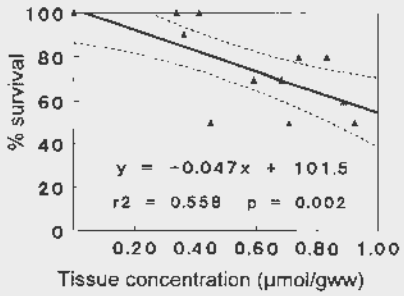

Fig. 2. Concentration of fluoranthene-equivalents in tissue of *Hyalella azteca* versus percent survival after 16 d of exposure to a range of concentrations of fluoranthene in sediment. Dashed lines represent 95% confidence intervals.

which lowest BSAFs were observed for trace level exposures (0.045–0.436), lowest BSAFs in the present experiments were observed for animals exposed to the highest sediment concentrations (0.129–0.170, Table 4). Maximum BSAFs were generally observed on day 30, in contrast to previous experiments where BSAFs declined slightly at the last time point. Average (SD) lipid content (on a dry weight basis) at the beginning of the experiment was 5.4% (0.9) for *H. azteca* (n = 4), and 25.4% (7.4) for *Diporeia* (n = 5). Average lipid content determined at the end of the experiment did not appear to change in relation to sediment fluoranthene concentration (Table 4), as was observed in some cases for *Diporeia* in previous experiments [7].

Sediment uptake clearance rates, which could only be calculated for *Diporeia* in these experiments, ranged from 0.108 g dry weight sediment cleared/g wet weight tissue/d for the highest sediment concentration to 0.608 g dry weight/g wet weight/d at an intermediate sediment concentration (Table 5). As was seen in previous experiments [7], uptake clearance rates were maximal at intermediate sediment concentrations (Table 5). Values for λ, the rate constant for the apparent reduction in the bioavailable fraction of fluoranthene in the sediment, ranged from 0.004 to 0.022 per day. Uptake for *H. azteca* did not fit any available models, including growth dilution models. No significant growth of *Diporeia* occurred over the course of the experiment, but *H. azteca* exhibited growth at all doses (Table 6). No dose-dependent effect on growth was apparent.

DISCUSSION

One goal of the present experiment was to test the ability of the EqP approach to predict the toxicity of sediment-associated hydrophobic contaminants. Equilibrium partitioning predicts that significant mortality will occur when the interstitial water concentration exceeds the water-only LC50. The 10-d LC50 for exposure of *H. azteca* to fluoranthene was previously determined to be 564 nmol/L, as the average of two independent experiments [19]. In the present experiment, interstitial water concentrations that were estimated from measured sediment and organic carbon concentrations (3,230 nmol/L), actually measured in interstitial water of centrifuged sediments (5,870 nmol/L, total fluoranthene), or estimated from the percent of the total measured concentration in the interstitial water that appeared to be freely dissolved (1,760 nmol/L) (Table 2) are as much as 10 times the 10-d water-only LC50. However, survival of *H. azteca* after 10-d sediment exposures in the present experiment was greater than 78% at all sediment concentrations (Table 3). Survival was reduced

Table 4. Percent sediment organic carbon, percent lipid content, and apparent steady state biota–sediment accumulation factors (BSAFs)

Organism	Mean measured sediment concn. (nmol/g dry wt.)	Mean (SD) % organic carbon, day 30 (n = 3)	Mean (SD) % lipid (dry wt.), day 30 (n)	Mean (SD) tissue concn., day 16 (n = 3) (μmol/g lipid)	Mean (range) apparent steady state BSAF, day 16 (n = 3)	Mean (SD) tissue concn., day 30 (n 5) (μmol/g lipid)	Mean (range) apparent steady state BSAF, day 30 (n 5)
Diporeia spp.	Control	ND[a]	27.2 (1.3) 2	ND	ND	ND	ND
	0.165	1.03 (0.05)	17.5 (6.5) 4	0.007 (0.002)	0.417 (0.320–0.526)	0.004 (0.001)	0.236 (0.174 0.327)
	607	1.10 (0.18)	24.9 (3.0) 3	71.0 (8.4)	1.33 (1.11–1.57)	63.7 (7.9)	1.28 (1.05–1.64)
	898	1.09 (0.07)	24.4 (4.3) 4	35.8 (14.5)	0.492 (0.265–0.691)	49.4 (11.4)	0.654 (0.481–0.754)
	2,270	0.98 (0.06)	24.4 (5.5) 5	77.2 (24.1)	0.346 (0.286–0.442)	91.5 (11.9)	0.424 (0.323–0.497)
	2,800	1.07 (0.09)	24.8 (2.9) 5	74.3 (4.8)	0.170 (0.134–0.212)	84.0 (9.4)	0.219 (0.157–0.248)
Hyalella azteca	Control	ND	7.5[b] (1.5) 4	ND	ND	ND	ND
	0.152	1.2 (0.12)	6.2 (1.1) 5	0.003 (0.0004)	0.180 (0.150–0.227)	0.003 (0.0005)	0.219 (0.172–0.270)
	563	1.03 (0.10)	5.3 (2.0) 5	25.8 (2.6)	0.474 (0.383 0.564)	29.2 (15.1)	0.564 (0.104–0.904)
	1,040	1.11 (0.10)	5.9 (1.2) 5	49.3 (4.1)	0.407[c] (0.402–0.412)	75.0 (15.3)	0.667 (0.571–0.854)
	1,850	1.12 (0.09)	6.6 (1.3) 2	40.4 (8.4)	0.216 (0.188 0.244)	66.9 (24.8)	0.360 (0.192–0.539)
	3,940	1.05 (0.17)	ND	38.3 (13.3)[d]	0.129 (0.095–0.147)	66.3 (28.2)[d]	0.206 (0.129–0.319)

[a] ND = not determined.
[b] Significantly different from day 0.
[c] Denotes n = 2.
[d] Used average lipid value of 6.3%.

after longer sediment exposures, but the results at later time points are not comparable to the 10-d water-only LC50.

Unlike the previous experiments in which survival of *H. azteca* remained high at later time points (>70%) [7], significant mortality was observed in the present experiment after longer exposures (16 or 30 d, Table 3). After 16 d, an LC50 of 3,550 nmol/g dry weight was determined, which corresponds to an estimated interstitial water concentration of 2,910 nmol/L, a value 4.9 times the previously measured water-only 10-d LC50 [19]. These results suggest that the EqP approach overpredicts fluoranthene sediment toxicity for *H. azteca* in this sediment and, in general, for the two Lake Michigan sediments studied [this study, ref. 7]. Lower than expected toxicity was also observed for this species with sediments contaminated with dieldrin [4]. The authors of that study suggested that sediment avoidance may have reduced exposure, but did not have body burden data to confirm their speculations.

For *Diporeia*, results of the present experiment do not contradict EqP predictions. No mortality was observed for this species in previous 10-d water-only exposures at concentrations up to the limit of fluoranthene's water solubility [19], and no significant mortality was observed in the present sediment exposures. In one of two previous experiments, however, significant mortality was observed for *Diporeia* after 10-d exposures to fluoranthene-spiked sediments [7]. Although mortality was even greater at later time points in that experiment (up to 84% on day 30), unexpectedly high mortality in the controls led the authors to speculate that factors other than fluoranthene exposure, such as seasonal differences in lipid content and age of the animals, were contributing to observed mortality. Significant mortality in *Diporeia* at later time points in both previous sediment experiments (up to 47 and 84%, respectively) are not comparable to LC50s established for 10-d water-only exposures and therefore do not contradict the EqP approach.

A second goal of the present experiment was to evaluate the ability of the CBR approach to predict toxicity of a narcotic compound such as fluoranthene. Mortality was expected to occur at body burdens in the range of 2 to 8 μmol/g wet weight tissue. In previous experiments, low mortality (<30%) was observed for *H. azteca*, which typically attained body burdens of less than 1 μmol/g wet weight in sediment exposures [7]. The authors suggested that *H. azteca* was exposed to overlying water, rather than pore water, because of its epibenthic lifestyle in that system. In addition, the ability of *H. azteca* to metabolize and rapidly eliminate fluoranthene (half-life = 4–6 h) may have contributed to its low body burden in comparison to *Diporeia*, which does not metabolize fluoranthene to an appreciable extent (half-life of 7–26 d) [19]. Similar results were observed in previous experiments, in which survival of *H. azteca* was greater than 90% after 10-d exposures to sediments with estimated and measured total interstitial water

Table 5. Uptake rate coefficients (g dry sediment/g organism/d) for *Diporeia* spp. and rate constants (d^{-1}) for the reduction in the bioavailable fraction of fluoranthene

Mean measured sediment concn. (nmol/g dry wt.)	Uptake rate constant (k_u)[a]	95% Confidence interval		λ (d^{-1})
		Lower	Upper	
0.171	0.143	0.082	0.203	0.022
587	0.608	0.451	0.765	0.003
799	0.373	0.284	0.462	0.011
2,160	0.201	0.159	0.243	0.002
4,250	0.108	0.091	0.126	0.004

[a] g dry wt. sediment/g wet wt. organism/d.

Table 6. Growth rate of *Hyalella azteca*, calculated from the regression of ln (wet weight) versus days of exposure

Mean measured sediment concn.[a]	Growth rate constant (d^{-1})	Standard error	r^2	(n)
1.52	0.043	0.006	0.743	(22)
563	0.043	0.005	0.779	(22)
1,040	0.052	0.006	0.786	(21)
1,850	0.045	0.007	0.710	(21)
3,940	0.040	0.004	0.806	(22)

[a] nmol/g dry wt.

concentrations of 1,650 and 569 nmol fluoranthene/L, respectively [7]. In the present experiments, maximum body burdens of fluoranthene-equivalents in *H. azteca* were slightly higher than 1 μmol/g wet weight after 30-d exposures at the three highest sediment concentrations, and a body burden of 1.10 μmol/g wet weight was associated with 50% mortality on day 16 (Fig. 2). However, rough estimates of critical body burdens measured for *H. azteca* after shorter, 10-d water-only exposures were higher, 3.6 to 5.6 μmol/g wet weight [19]. Because *H. azteca* is known to metabolize fluoranthene [19] and other PAHs [29] to more polar and potentially more toxic intermediate metabolites, we hypothesize that fluoranthene is not acting solely as a narcotic compound in this species. At present, only a general estimate of the CBR for fluoranthene of 1 to 5.6 μmol fluoranthene-equivalents/g wet weight can be made for this species.

Although *Diporeia* attained higher maximum body burdens in the present experiment (up to 5.9 μmol/g wet weight) than was observed in previous experiments (approx. 4 μmol/g wet weight) [7], less mortality (≤30%) was observed in the present experiment (Table 3) than in previous experiments (up to 86% mortality after 30 d). Rough estimates of the CBR associated with 50% mortality in the previous fluoranthene sediment study ranged from 2.7 to 6.5 μmol/g wet weight, for 10- and 30-d exposures, respectively, in two separate experiments [7]. However, as previously discussed, factors other than exposure to fluoranthene contributed to the mortality observed in the first experiment, and the actual LD50 for exposure to fluoranthene was probably closer to 6.5 μmol/g wet weight. Other studies with *Diporeia* have estimated the body burden of PAHs required to produce a toxic effect to be in the range of 6 to 9 μmol/g wet weight [5,6]. The actual critical body burden for *Diporeia* appears to be in the high end of, or perhaps exceeds, the expected range of values, 2 to 8 μmol/g wet weight, for narcotics [8].

Although the exact mechanism underlying the narcotic effect of hydrophobic compounds remains unknown, the site of action for narcotic chemicals is thought to be the lipid membrane layer [9]. Normalization of tissue concentrations of chlorinated hydrocarbons to lipid content in the fathead minnow substantially reduced variability in estimates of critical body burden [13]. The authors of that study estimated that for a fish with a 5% fat content and a critical body burden of 2 to 8 μmol/g, the critical membrane burden would be 40 to 160 μmol/g lipid. For *Diporeia,* with an average lipid content in this experiment of 24% (dry weight), and an estimated critical body burden of 6 to 9 μmol/g wet weight (~22–33 μmol/g dry weight) from previous experiments [5 7], the lipid-normalized critical body burden would be 92 to 138 μmol/g lipid. In the present experiment, the lipid-normalized tissue concentration did not exceed 77 μmol/g on day 16 (Table 4). Therefore, low mortality observed for *Diporeia* at that time point would be expected on the basis of its lipid-normalized body burden. Higher body burdens at day 30 (up to 92 μmol fluoranthene/g lipid), which are at the low end of the range of the expected lipid-normalized CBR for this species (92–138 μmol/g lipid), might have been expected to result in more mortality than was observed at that time point (<16%, Table 3). Nonetheless, these results suggest that lipid-normalization of body burdens may prove to be a useful refinement of the CBR approach for narcotic compounds. Values for lipid-normalized tissue concentrations for *H. azteca* are included for comparison (Table 4), but because this species is known to metabolize fluoranthene as noted in the above discussion, some fraction of its body burden has been transformed to potentially less narcotic metabolites with more specific modes of action, and application of the CBR approach to lipid-normalized values may be less valid.

CONCLUSIONS

For *H. azteca,* the EqP approach consistently overpredicted the toxicity of fluoranthene in Lake Michigan sediments. For *Diporeia,* EqP either underpredicted or appropriately predicted the response. The CBR approach is a useful complement to the EqP approach for assessment of the potential toxicity of sediment-associated contaminants. Further testing with a variety of species and compounds will be important to fully assess the validity of these approaches. Interspecific differences in ability to metabolize compounds of concern may be especially critical to a thorough understanding of the potential for toxicity associated with narcosis.

Acknowledgement—This work was funded in part through interagency agreement DW13936659-01-2 between the U.S. EPA and the National Oceanic and Atmospheric Administration (NOAA). Although this work was supported by the U.S. EPA and NOAA, it does not necessarily reflect the views of the agencies; no official endorsement should be inferred. Mention of trade names does not constitute endorsement or recommendation for use. This publication is a result of work sponsored by the Cooperative Institute for Limnology and Ecosystems Research under cooperative agreements from the Environmental Research Laboratories, National Oceanic and Atmospheric Administration, and the U.S. Department of Commerce under cooperative agreement NA90RAH00079. We wish to thank F. Lo and E. Tigue for excellent technical assistance and P. Van Hoof and J. Hsieh for assistance with GC-MS. We also wish to thank C. Ingersoll and E. Greer for the gift of the *H. azteca,* and D. Gossiaux and the crew of the R/V *Shenehon* for collection of *Diporeia.* This is GLERL contribution 1003.

REFERENCES

1. **Di Toro, D.M., et al.** 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ. Toxicol. Chem.* **10**:1541–1583.
2. **Swartz, R.S., D.W. Schults, T.H. DeWitt, G.R. Ditsworth** and **J.O. Lamberson.** 1990. Toxicity of fluoranthene in sediment to marine amphipods: A test of the equilibrium partitioning approach to sediment quality criteria. *Environ. Toxicol. Chem.* **9**:1071–1080.
3. **Hoke, R.A., G.T. Ankley, A.M. Cotter, T. Goldenstein, P.A. Kosian, G.L. Phipps** and **F.M. VanderMeiden.** 1994. Evaluation of equilibrium partitioning theory for predicting acute toxicity of field-collected sediments contaminated with DDT, DDE, and DDD to the amphipod *Hyalella azteca. Environ. Toxicol. Chem.* **13**:157 166.
4. **Hoke, R.A., P.A Kosian, G.T. Ankley, A.M. Cotter, F.M. Vandermeiden, G.L. Phipps** and **E.J. Durhan.** 1995. Check studies with *Hyalella azteca* and *Chironomous tentans* in support of the development of a sediment quality criterion for dieldrin. *Environ. Toxicol. Chem.* **13**:435–443.
5. **Landrum, P.F., B.J. Eadie** and **W.R. Faust.** 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia* spp. *Environ. Toxicol. Chem.* **10**:35–46.
6. **Landrum, P.F. W.S. Dupuis** and **J. Kukkonen.** 1994. Toxicity and toxicokinetics of sediment-associated pyrene in *Diporeia* spp.: Examination of equilibrium partitioning theory and residue based effects for assessing hazard. *Environ. Toxicol. Chem.* **13**: 1769–1780.
7. **Kane Driscoll, S.B., G. Harkey** and **P.F. Landrum.** 1997. Accumulation and toxicokinetics of fluoranthene in sediment bioassays with freshwater amphipods. *Environ. Toxicol. Chem.* **16**: 742–753.
8. **McCarty, L.P.** and **D. Mackay.** 1993. Enhancing ecotoxico-

logical modeling and assessment: Body residue and modes of toxic action. *Environ. Sci. Technol.* **27**:1719–1728.
9. **Van Wezel, A.P.** and **A. Opperhuizen.** 1995. Narcosis due to environmental pollutants in aquatic organisms: Residue based toxicity, mechanisms, and membrane burdens. *Crit. Rev. Toxicol.* **25**:225–279.
10. **Van Hoogen, G.** and **A. Opperhuizen.** 1988. Toxicokinetics of chlorobenzenes in fish. *Environ. Toxicol. Chem.* **7**:213–219.
11. **McCarty, L.P., D. Mackay, A.D. Smith, G.W. Ozburn** and **D.G. Dixon.** 1992. Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: Neutral narcotic organics. *Environ. Toxicol. Chem.* **11**:917 930.
12. **Sijm, D.T.H.M., M. Schipper** and **A. Opperhuizen.** 1993. Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological endpoint. *Environ. Toxicol. Chem.* **12**:1117–1127.
13. **Van Wezel, A.P., D.A.M. de Vires, S. Kostense, D.T.H.M. Sijm** and **A. Opperhuizen.** 1995. Intraspecies variation in lethal body burdens of narcotic compounds. *Aquat. Toxicol.* **33**:325–342.
14. **Pawlisz, A.V.** and **R. Peters.** 1993. A test of the equipotency of internal burdens of nine narcotic chemicals using *Daphnia magna*. *Environ. Sci. Technol.* **27**:2801–2806.
15. **Pawlisz, A.V.** and **R.H. Peters.** 1995. Effects of sublethal exposure on lethal body burdens of narcotic organic chemicals in *Daphnia magna*. *Environ. Sci. Technol.* **29**:613 -621.
16. **U.S. Environmental Protection Agency.** 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-94/024. Office of Research and Development, Duluth, MN.
17. **American Society for Testing and Materials.** 1995. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. E-1383-94a. In *Annual Book of ASTM Standards, Volume 11.05*. Philadelphia, PA, pp. 802–834.
18. **U.S. Environmental Protection Agency.** 1993. Sediment quality criteria for the protection of benthic organisms: Fluoranthene. EPA 882-R-93-012. Office of Water and Office of Research and Development, Washington, DC.
19. **Kane Driscoll, S.B., P.F. Landrum** and **E.Tigue.** 1997. Accumulation and toxicokinetics of fluoranthene in water-only exposures with freshwater amphipods. *Environ. Toxicol. Chem.* **16**:754–761.
20. **Harkey, G.A., P.L. Van Hoof** and **P.F. Landrum.** 1995. Bioavailability of polycyclic aromatic hydrocarbons from a historically-contaminated sediment core. *Environ. Toxicol. Chem.* **14**:1551–1560.
21. **Ditsworth, G.R., D.W Schults** and **J.K.P. Jones.** 1990. Preparation of benthic substrates for sediment toxicity testing. *Environ. Toxicol. Chem.* **9**:1523 1529.
22. **Eadie, B.J., P.F. Landrum** and **W. Faust.** 1982. Polycyclic aromatic hydrocarbons in sediments, porewater, and the amphipod *Pontoporeia hoyi* from Lake Michigan. *Chemosphere* **9**:847–858.
23. **Gardner, W.S., W.A. Frez, E.A. Chichocki** and **C.C. Parish.** 1985. Micromethod for lipid analysis in aquatic invertebrates. *Limnol. Oceanogr.* **30**:1099–1105.
24. **Zumwalt, D.C., F.J. Dwyer, I.E. Greer** and **C.G. Ingersoll.** 1994. A water-renewal system that accurately delivers small volumes of water to exposure chambers. *Environ. Toxicol. Chem.* **13**:1311 1314.
25. **Harkey, G.A., S. Kane Driscoll** and **P.F. Landrum.** 1997. Effect of feeding in 30-day bioaccumulation assays using *Hyalella azteca* in fluoranthene-dosed sediment. *Environ. Toxicol. Chem.* **16**:762–769.
26. **Landrum, P.F., S.R. Nihart, B.J. Eadie** and **W.S. Gardner.** 1984. Reverse-phase separation method for determining pollutant binding to Aldrich humic acids and dissolved organic carbon of natural waters. *Environ. Sci. Technol.* **18**:187–192.
27. **Sokal, R.R.** and **J.F. Rohlf.** 1981. *Biometry*, 2nd ed. W.H. Freeman, New York, NY, USA.
28. **Landrum, P.F.** 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi*. *Environ. Sci. Technol.* **23**:588–595.
29. **Landrum, P.F.** and **D. Scavia.** 1983. Influence of sediment on anthracene uptake, depuration, and biotransformation by the amphipod *Hyalella azteca*. *Can. J. Fish. Aquat. Sci.* **40**:298–305.